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THE USE OF ACTIVE HUMAN SERUM IN THE SERUM DIAGNOSIS OF SYPHILIS.*

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Since Wassermann's original communication in 1906 of a method for the diagnosis of syphilis by means of the identification of specific bodies present in the serum of luetic individuals, many unnecessary changes and several important and valuable improvements in the methods of technic have been brought forward. Of the modifications suggested and proven valuable that of the more precise determination of the active principle of the antigen has been universally recognized. Soon after the original communications it was shown both by Wassermann himself and by others (Levaditi and Landsteiner) that extracts of the spirochete as prepared from syphilitic livers were not essential constituents, but that lipoid bodies capable of extraction, both from the liver and from other organs, preferably the human heart, were more potent. To Noguchi,¹ in particular, recognition is due for the demonstration of the rôle played by the phosphated group of lipoids in the binding of complement in the presence of the so-called syphilitic antibodies.

In addition to this standardization, as it were, of the antigenic properties of the tissue extracts, several real and important modifications of the Bordet reaction as utilized by Wassermann have been published. There is no necessity to describe, *in extenso*, the details of all the various reactions, but a review of the more important, and criticisms based on personal observation of those which appear to deserve such criticism, will be made. Excluding the use of antigens prepared in different ways, and here it may be insisted that the chief reliability of the reaction no matter how carried out depends on the specificity of the antigen, there remain two essential manners in which the performance of the reaction may differ, one of these being of the greatest possible moment, the

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¹ *Jour. Exp. Med.*, 1911, 13, p. 43.

other being of comparatively little importance, granted certain fundamental principles are complied with. I refer to the use of human or guinea-pig serum for complement and the employment of red-blood cells from different animals or from man. Much has been written by different authors advocating the use of some particular hemolytic series. Thus Wassermann following Bordet considers that sheep corpuscles and a specific hemolysin should be employed; still more emphatically Noguchi states that human corpuscles and a corresponding amboceptor must be used in order to get reliable results. Again others suggest the use of corpuscles from the hen and other animals.

It must be evident to all that the corpuscles used in the second part of the reaction are merely indicators and that, so long as the serum used as hemolysin can be exactly standardized and its amboceptor is capable of readily combining with complement, it is of little importance what type of red-blood cell be used, granted the accidental presence of bodies potent to increase or diminish the activity of the amboceptor can be excluded.

In general the methods for carrying out the reaction may be divided into three groups, namely (1) those in which both complement and the hemolysin are utilized from serum other than the human, usually guinea-pig serum for the former body and immunized rabbit serum for the latter; (2) those in which the normal complement content of the human serum is utilized, the hemolytic specific body being derived from rabbit serum as in the first group; and (3) those methods in which both complement and hemolysin are obtained from the serum tested.

It is apparent that one or other of these methods, if not the best under all circumstances, must be more adaptable at certain times. It will be the aim to present as clearly and fairly as possible the relative advantage of the various systems and though recommending strongly one method in particular, to appreciate the limitations and sources of error which must be considered in the use of this method. Before discussing the relative merits of the methods of Wassermann and Noguchi whose systems fall into the first group, that of Stern whose method consists in the use of normal human complement and rabbit serum hemolysin, and the methods of Hecht and Tschers-

nogubow and the modification of the latter as employed by the author, we will consider what characteristics a system of serum diagnosis should have in order to be valuable, and then note in how far the different systems comply with the essentials laid down.

In order of importance these essentials are assumed to be: (1) theoretical and experimental exactness, (2) practical correctness, (3) delicacy, (4) simplicity of procedure, (5) adaptability to use at all times and in all cases.

There being very little doubt but that methods of the third group are more simple, let us consider first the theoretical basis upon which the methods rest, discussing last the practical value and adaptability of the procedure as advocated by Tschernogubow and modified by the author.

Theoretically the Bordet-Gengou reaction depends on the fixation or binding of complement by means of an antigen and a specific antibody. That red-blood cells are employed with a specific hemolysin as an indicator is merely a matter of convenience such as the use of phenolphthalein or litmus in titration. Two things are, therefore, essential: first, since the reaction as used in the diagnosis of syphilis is a quantitative one, the complement employed must be of known quantity and, secondly, it must be susceptible to fixation. In the second place, since we know that in the presence of a larger quantity of hemolytic amboceptor a smaller quantity of complement is sufficient to produce dissolution of the blood cells, and in particular owing to the fact that in the presence of a sufficiently potent hemolysin it is possible for the fixed complement to be deviated, it is important that the hemolytic amboceptor present be also of known quantity.

In the original method as advanced by Bordet and Gengou and followed by Wassermann, guinea-pig complement was employed and measured according to the quantity of serum used, it being taken for granted that the complement content of such serum is constant. That such serum is tolerably constant with reference to its complement content we are prepared to admit; that it is not absolutely constant has been repeatedly proven both by the author and others. An article is now in preparation dealing with the variations in complement content in the serum of man and animals as

influenced by age, health, feeding, etc., and also the changes in the quantity of complement following removal from the body under different circumstances of temperature, etc. This subject will not, therefore, be taken up, *in extenso*, in this paper; suffice to say that I have found with others—Clarke,¹ Hecht,² König,³ Tschernogubow,⁴ and Stern and Noguchi⁵—that the complement content of serum from the guinea-pig is liable to vary at times, even in fresh serum, four to eight hours old, sometimes being double that in other sera of the same age and kept under similar conditions. Complement as present in the guinea-pig serum alters after its removal from the animal, gradually increasing in amount if kept for a short time at incubator temperature and thence in the ice chest, remaining for the first 24 to 48 hours practically constant and then, at first slowly, later more rapidly, deteriorating. The changes in complement content following removal of the blood are similar in both human and guinea-pig serum and the influence of the conditions under which the blood is kept appear to affect human complement in practically the same way as that derived from the guinea-pig. In common with Noguchi I have found that certain sera lose their complementary activity more rapidly than others. I believe, however, that as a rule accidental factors such as contamination of the serum by bacteria or protein bodies influence this variation more than any inherent property of the serum.

In 500 tests made on the complement content of human serum the quantitative constancy of this body has been proven. In all sera measured after a lapse of less than 36 hours, and this refers to over 90 per cent of those tested, in only 20 cases have demonstrable variations from the normal been detected, and even in those cases, as will be shown later, this difference is probably more apparent than real. Truly speaking, complement as such has not been estimated owing to technical difficulties not usually appreciated; what has been really tested has been the combined action of complement and the hemolysin content of human serum on guinea-pig corpuscles. For this purpose serum diluted in the proportion of 1-10 in salt solution has been added in quantities (of the diluted material) of 1 c.c., 5 c.c., 0.35 c.c., and 0.25 c.c. to 0.25 c.c. of a 5 per cent suspension of washed guinea-pig corpuscles. The mixture was placed in the incubator and read at the end of 45 minutes, at which time it has been found that the end reaction is present. The rule has been that the quantities representing 0.1 c.c., 0.05 c.c., and 0.035 c.c. of the serum uniformly cause complete lysis whereas the

¹ *Jour. Infect. Dis.*, 1910, 7, p. 476.

² *Wien. klin. Wchnschr.*, 1908, 21, p. 1742; *ibid.*, 1909, 22, p. 338.

³ *Deut. med. Wchnschr.*, 1910, 36, No. 11.

⁴ *Ibid.*, 1909, 35, p. 668.

⁵ *Jour. Exp. Med.*, 1911, 13, p. 69.

quantity of 0.025 c.c. of serum has usually proven insufficient. In 12 cases 0.5 c.c. was insufficient and in five others 0.35 c.c. was found incapable of completing lysis. In all the former cases the serum was from syphilitic individuals. It is thought that the reason for the smaller quantity of complement was the contamination of the serum at some time in the process of handling with lipoids potent to bind some of the complementary bodies. That this error may and does occasionally take place is undoubtedly so, as I have several times noted such a false result in the results of those working in our laboratory who have employed in the transfer of the serum pipettes usually used for measuring the antigen. The importance of this liability to error will be referred to again in comparing the relative value of different methods. Contamination of the serum with protein bodies resulting in the non-specific proteotropic reaction may also result in the binding of complement, rendering it unavailable for further use. The majority of the cases in which lysis was not complete with 0.05 c.c. of serum occurred within a period lasting a couple of weeks. I noted that many of the tubes used for diluting the serum were somewhat cloudy. On washing all the test tubes used in the reaction in alcohol and ether, I found that such lack of hemolysis was prevented and since making it a routine practice to treat all tubes in this manner I have had no further false controls.

Attempts were made to test the complement content in the human serum by itself by means of a hemolytic series consisting of rabbit serum and human red-blood cells. These efforts, however, proved futile since even in the presence of 0.15 c.c. of human serum (8 hours old) absolutely no hemolysis of 1 c.c. of human corpuscle according to Noguchi's suspension (1 drop to 4 c.c.) occurred when twice the quantity of rabbit serum necessary to produce complete hemolysis when acting with 0.04 c.c. of guinea-pig serum was added. As 0.035 c.c. of this human serum was sufficient to hemolyze 0.25 c.c. of a 5 per cent suspension of guinea-pig serum, a much larger number of corpuscles, it was thought strange that such a result should be obtained, and an effort was therefore made to measure the human complement content by replacement of this body after destruction by time—e.g., one week in the ice chest, as this method has been found to weaken the amboceptor content much less than heat, while it practically allows the complete deterioration of complement. By this means it was found that in order to completely hemolyze within two hours the standard quantity of guinea-pig corpuscles the presence of 0.1 c.c. of complement was necessary. It will be noted that in this experiment three times the quantity of amboceptor necessary to cause complete lysis with 0.035 c.c. of human complement was present. In the presence of three quantities of amboceptor less than half the quantity of complement necessary should suffice, whereas in this case three times as much serum was necessary, or six times what would have been needed if human serum had been used. Repeated experiments proved that this inactivity on the part of human complement when treated with rabbit hemolysin and a similar inactivity on the part of guinea-pig complement with human amboceptors is constant. Owing, therefore, to the inactivity of the human hemolysin in the presence of guinea-pig complement and the apparent lack of activating power of human complement in the presence of rabbit hemolysin, it was considered more exact to estimate the joint action of these two bodies in the human serum, complement, and hemolysin, rather than the activating value of human complement upon immune bodies in foreign sera. It seemed unlikely also that in so large a series of reactions the coincidence of a greater complement and a smaller amboceptor (hemolytic) content would be constant.

The estimations referred to have been made in normal individuals, and in those suffering from chronic diseases, including leprosy, tuberculosis, carcinoma, syphilis, cardiorenal disease, etc., excepting those in a dying condition. In acute febrile conditions, especially in the terminal stages, complement may be diminished in quantity, but such cases are rarely the subject of syphilitic reactions and such insufficiency can hardly militate against the value of the reaction. As is well known the absence of amboceptor against foreign corpuscles in sucklings renders the use of any method of the third series useless with infants less than one and a half to two years old.

It has, therefore, been proved that in so far as the constancy of human complement is concerned it is equally as reliable as that from the guinea-pig. It has further been proved that human amboceptors, of the hemolytic order at least, are more active in the presence of human complement than that derived from the serum of the guinea-pig.

A further feature, however, theoretically renders the use of unheated human serum more valuable than that of serum heated to 56° C. as is done in the inactivation of complement. I refer to the fact that not only is the complementary body destroyed by such a temperature but at least one half of the amboceptor content is also rendered useless. Thus unheated serum will possess a larger quantity of the specific body being sought, rendering its recognition more easy and permitting a finer differentiation quantitatively.

Since no foreign serum is added at any time the possibility of a non-specific fixation, the result of the proteotropic reaction, is excluded if the antigen used is prepared in such a manner as to be free from such material. Hence no danger arises in the use of active serum in which the proteotropic bodies are not destroyed.

As Noguchi has pointed out, if a foreign serum such as that from the rabbit is to be used for its hemolysin content the titre of this serum must be as high as possible in order that a minimal amount of foreign protein substance may be added and thus obviate the possibility of the proteotropic non-specific reaction.

The possibilities for error in the original Wassermann reaction

arise from the following facts: (1) The inactivation of the tested serum results in the destruction of most of the syphilitic antibody; (2) the guinea-pig serum used for complement may vary in its complement content; (3) human serum contains a certain quantity of antish sheep hemolysin, thus rendering the standardization of the hemolytic amboceptor difficult.

Noguchi's modification using fresh serum does away with the first objection, in the same way the use of a human hemolytic series excludes the third liability to error, but both of these advantages are replaced by other possibilities of error almost equally important. The use of active serum renders possible a reaction between the proteotropic bodies and the protein in either or both of the foreign sera added. Thus a positive instead of a negative reaction may occur with normal sera. This possibility of error, it is only fair to state, has not been found to be of practical importance especially if the serum from the rabbit be of a high hemolytic value.

A more influential factor is that of the antihuman hemolysin present in the guinea-pig serum used as complement. I have found that two and one-half times the quantity of serum used by Noguchi is usually sufficient to completely hemolyze the red cells used in the absence of other specific lysins. The serum from one pig was constantly found to hemolyze the entire quantity of cells in as small quantities as 0.02 c.c. This objection to the method advised by Noguchi is of importance, but in the author's experience the technical difficulties arising from the use of such small quantities of material and the difficulty of reading reactions when such a small number of red cells are employed, especially as they are agglutinated by the agglutinin present in the hemolytic serum, are even greater.

Hecht in the method usually associated with his name makes use of the normal human complement content and for hemolytic amboceptor depends on the antish sheep amboceptor in human serum. This antish sheep lysin is, however, present in smaller quantities than that against guinea-pig corpuscles. Thus the number of free complement units is smaller and the delicacy of the reaction interfered with to this extent. The poor activating power of human

complement of amboceptor derived from the rabbit practically prevents the use of the method advocated by Stern.

In 1909 Tschernogubow¹ recommended the employment of the natural complement in human serum as well as the natural lysin for guinea-pig corpuscles. He recommended the use of 0.1 c.c. of serum with an appropriate quantity of antigen in 1 c.c. of salt solution. To this mixture after one hour is added 0.25 c.c. of a 5 per cent suspension of guinea-pig corpuscles. Although Tschernogubow lays stress on the necessity for exact controls he does not state definitely the manner in which they are carried out. I believe then the reason why Tschernogubow's method has not been used more widely, especially in this country, is the fact that it is somewhat difficult to understand from reading his articles exactly how the reaction is performed by him. There can be no doubt but that many, if not all, of the unfavorable criticisms have been made by writers who have not conscientiously attempted to determine the value of the method but have accepted too literally the prevalent idea that the hemolytic body and complement in human serum varies. The smaller unit and consequent larger number of units of complement present in human serum when the anti-guinea-pig corpuscle lysin is employed renders the small variations in complement negligible, whereas in the Hecht method even slight variations may prove very important.

The technic employed by the author and which has been found to be very satisfactory from all points of view is as follows.

Two c.c. of blood are removed in the usual manner from a vein at the elbow. This is placed in a small test tube and kept in the incubator (37° C.) for 2 hours, and then placed in the ice chest. It has been found that such serum will remain reliable for at least 24 hours, usually for 48, and rarely for 72 hours. The most constant results are, however, obtained if sera either 4 to 6 hours or 20 to 28 hours old are employed. Thus the serum collected on two successive days can be tested at the same time.

Four-tenths c.c. of the serum is placed in a small test tube and 4 c.c. of salt solution added. Of the diluted material six test tubes are filled as follows:

	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6
Serum	1 c.c.	1 c.c.	0.5 c.c.	0.35 c.c.	0.25 c.c.	
Antigen	1 c.c.	1 c.c.	1 c.c.

The antigen used is prepared according to Noguchi's acetone insoluble method, thus obviating the non-specific proteotropic reaction. The lipoids are kept dissolved

¹*Op. cit.*

in alcohol and diluted 1-30 in salt solution for use, the dilution necessary varying, of course, with the potency of the antigen.

The mixture is placed in the incubator for one hour, at the end of which period 0.25 c.c. of a 5 per cent suspension of washed corpuscles is added to all tubes and 0.5 c.c. to tube 2. The tubes are replaced in the incubator for 30 or 45 minutes and can be read immediately or left until the following day. In the hot summer climate in New Orleans the bacterial growth is however liable to obscure the result if the reading is postponed.

Tubes 3, 4, 5, and 6 are of course controls, the last noting the absence of hemolytic action on the part of the antigen and need not be repeated with each series. The preparations containing 0.5 c.c. of the serum and that containing 0.35 c.c. will be found completely hemolyzed, whereas the one containing the smallest quantity will contain an insufficient amount of hemolysin and complement to cause complete lysis.

If the first tube shows complete inhibition of hemolysis it is evident that the Wassermann antibody is potent to fix at least five units of complement. As the units are small the reaction is delicate and the greatest reliance can be placed on this positive result. If the first tube shows hemolysis and the second tube also, a distinctly negative reaction may be reported. If the first tube is hemolyzed and the second cloudy it is known that the amount of antibody present in the suspected serum is insufficient to fix nine units, although enough is present to fix two units. Such a reaction has been called by the author weakly positive. It is extremely delicate and at the same time very easy to read. So delicate in fact, that its usefulness is more or less limited, being given by a certain number of conditions other than those frankly syphilitic. This weak reaction is, however, of the greatest value in the examination of serum used as a control to the efficacy of treatment. Following a course of treatment either with mercury or arsenobenzol the persistence of such a reaction is, in the author's experience, presumptive of the insufficiency of treatment, similarly the development of such a reaction following its disappearance as the result of treatment suggests at least the necessity for careful control of the patient by means of repeated reactions. At present the author's experience with reference to the value of the control of therapeutic measures by means of serum reactions is too limited for a definite statement of opinion. A number of cases have been followed during the past 15 months and will be reported in detail in a future publication. Suffice it to say that repeated tests are

very valuable and the importance of the weak reaction shown by this method is of the greatest possible value. The reaction, being easily read, can be reported with greater confidence than one performed by either the original Wassermann method or the Noguchi modification.

Increased delicacy in any reaction is usually considered of great value. As a rule, however, in discussing the Wassermann reaction a proper appreciation of the value of this quality is not present. Since the reaction is quantitative even more than qualitative, it is quite possible to make the identification of complement binding bodies so delicate that for practical purposes the whole reaction may be rendered useless. In general a reaction in which the complement units are individually small and collectively numerous renders it possible to estimate more exactly the antibody content of the serum examined. To obtain this quantitative reaction by the author's method it is necessary to perform two tests instead of estimating the proportionate hemolysis in a single tube. This is, however, a simple matter and the ease with which the reaction is read more than compensates. As a matter of fact 50 per cent of cases giving the weak reaction by this method are negative by the Noguchi or Wassermann system, and, as has been shown, this weak reaction is extremely valuable in estimating the efficacy of treatment.

No method which necessitates the inactivating of the tested serum can ever give as definite fixations of complement as one in which the so-called syphilitic antibody has not been depreciated by heat. Not only does the greater amount of antibody and the smaller complement unit, together with the large number of units, increase the delicacy of the reaction, but the increased activity of human amboceptors in the presence of human complement as compared with their action with guinea-pig complement results in a more complete and rapid fixation of the complementary body. The increased activity of the hemolytic amboceptor with human complement also allows the hemolytic reaction to proceed more rapidly, thus the readings can be made after the lapse of a shorter period, which in many instances is a decided advantage.

Although for practical diagnostic and even prognostic purposes

the intensity of the reaction, so long as it be sufficiently marked to be capable of binding complement to the extent of inhibiting completely hemolysis by the method described, is of comparatively little importance, such quantitative determinations may be carried out by adding to the tested serum quantities of normal serum. This can best be carried out by using a fixed quantity, say 0.1 c.c., of normal serum and adding to it varying quantities of the serum to be tested.

Naturally the real proof of the value of any reaction depends upon its practical correctness, for, unless the results obtained are valuable, no amount of theoretical reasoning as to the probable value need be considered. Not only, however, is the method recommended in this paper theoretically superior to other systems, but practically it has been found to be more correct.

Of 626 tests 381 gave positive results, 190 negative, and 55 weakly positive as shown in the following summary:

	Positive	Weakly Positive	Negative
Primary syphilis less than 3 weeks...	0	2	5
Primary syphilis over 3 weeks	17	0	0
Secondary syphilis active.....	60	0	1
Tertiary syphilis active.....	115	3	2
Distinct history, but no active syphilis	78	11	6
Treated cases (mercury)	0	15	22
General paresis and tabes.....	13	9	3
Aneurism.....	4	0	2
Congenitally syphilitic children.....	25	2	0
Mothers of syphilitic infants.....	21	1	1
Cases for diagnosis.....	30	6	64
Leprosy.....	18	3	7
No indications of syphilis.....	0	3	77
	<hr/> 381	<hr/> 55	<hr/> 190

It is not my intention in this paper to discuss the value of the serum reaction in syphilis as a whole. The importance of the reaction is being appreciated and it is taking its proper place in the diagnosis of the disease and in controlling the efficiency and sufficiency of treatment. The results here tabulated compare favorably with those obtained by other observers with other methods, the absence of positive findings in negative cases, in so far as the clinical diagnosis was certain, being absolute.

Among the cases classified as "Cases for Diagnosis" are included those cases in which no history or at most a very indefinite history of infection could be determined. For the most part, among the positive cases in this series the serum reaction was accepted by the clinician as probably correct. Three, however, occurred in patients in which it was supposed active destruction of brain tissue was going on as a result of tumor or hemorrhage. That the serum from a certain number of cases of brain tumor gives a positive Wassermann reaction has been shown by various authors, and the author is at present engaged in experimental work with Dr. R. Van Wart in the hope of proving the cause of this reaction.

Of undoubted syphilitic cases, other than tabes and aneurism and those who had received prolonged and energetic treatment, only three gave negative reactions. One of these, a case of late secondary lues with mucous patches from which the treponema was identified, gave an absolutely negative result. The cause in this instance may have been accidental, but as the patient could not be found in order to repeat the reaction no explanation can be offered. The remaining two cases were patients suffering from glossitis and leukoplakia, in one instance 18 and in the other 29 years after the original infection, no more marked lesions having been present at any time.

The single case in which the mother of a syphilitic child gave a negative reaction is an extremely interesting one. Twins, three months of age, were brought to the clinic of Dr. DeBuys at the Touro Infirmary: one was an undeveloped scrawny child, the other apparently healthy. Reactions performed by the Noguchi method showed the serum of the sick infant to contain complement binding bodies in the presence of the lipoids, whereas the other child's serum was normal. The result was unlooked for and the test was, therefore, repeated and the serum of both mother and father was also tested. The original result in so far as the children were concerned was again obtained. The mother's serum proved normal while the father gave a most pronounced reaction. Two weeks after the reactions were performed a typical syphilitic rash appeared on the sick twin.

The cases of tabes and general paresis examined, though not

numerous, resulted in a higher percentage of positive reactions than has been usually reported. It is the author's belief that one important reason for the number of negative reactions given by tabetic sera is that prolonged mercurial treatment, such as these patients usually undergo, results in the arrest of the syphilitic process.

In conclusion I wish to state that of about 100 cases controlled by the Noguchi system in no instance was a negative result obtained by the Tschernogubow method with serum reacting positively to the other series, whereas three undoubted syphilitics were positive by the former where by the latter method complete hemolysis occurred. In addition to laying stress on the value of Tschernogubow's original method I wish to recommend the use of the more delicate reaction obtained by doubling the number of corpuscles added, especially in cases in which the efficacy of treatment is being determined. The author's method also of controlling the complement and hemolytic content of the tested serum is simpler and more exact than that described by Tschernogubow.

It will be noted that of a total of 28 cases of leprosy examined, 21 were found to have bodies present in the serum capable of fixing complement in the presence of the Wassermann antigen. As pointed out in a previous communication by Duval and myself,¹ all of those cases failing to react with the lipoids also failed to bind complement when treated with leprosy bacilli. All these cases, moreover, were considered inactive or cured by the clinical observers. It is our belief as expressed in the paper referred to that all cases of leprosy should, and most cases do, present in their blood Wassermann antibodies. The results published by various authors have varied from 40 to 80 per cent positive. For the most part, comparatively little note has been made of the stage of the disease except that many more positive reactions have been reported in the tubercular form than in those of purely so-called anesthetic types. It is the latter class of cases in which the greatest difficulty arises in stating whether or not the disease is arrested or cured. As stated in our previous communication we believe that not only can the cure of syphilis be controlled by the serum reaction but that the same may be stated with regard to leprosy.

¹ *Arch. Int. Med.*, 1911, 7, p. 230.

The last criterion by which the value of any diagnostic reaction can be judged is its universal application or, in lieu of this, a comprehensive knowledge of the factors rendering its use untrustworthy. Two important exceptions to the general adaptability of this reaction described in this paper are, first, the uselessness of the method without some modification, in infants, and, second, the necessity for using fresh serum—less than 48 hours old—since it is found that after this time the complement content is so liable to great variations that it is unsafe to employ the serum in the active state.

Why there should be an absence of hemolysin in the serum of sucklings is not apparent, but in all probability it is due to the fact that no animal cells being included in the dietary of the individual, no receptors capable of combining with foreign-tissue cells are necessary and are, therefore, not produced. The fact, however, is well established, having been pointed out by Bauer,¹ Hecht, and others several years ago.

Since the reaction described by Tschernogubow and advocated by the author cannot be used in all cases some other method must occasionally be employed. It is possible by means of normal human serum to supply the complement and amboceptor after inactivation of the serum to be tested. This method, however, is not recommended since the possibility of a non-specific reaction is increased, and, although good results have been obtained in the 15 cases so tested, I do not consider the method theoretically good. For such cases it has been my practice to make use of the original Wassermann technic, using human corpuscles and their specific hemolysin.

The method described is not recommended for use except by those working in laboratories and in institutions where a large number of reactions are carried out. Owing to its inexpensiveness, the small amount of time necessary to make the test, and the absence of reagents which are usually prepared with more or less difficulty, it places this important diagnostic aid within the reach of the clinician in a manner that would be impossible in a similar amount of time by any other method. If the serum to be examined is procured and kept as described the results will be uniformly good, and the time taken up in the performance will be greatly reduced.

¹ *Berl. klin. Wchnschr.*, 1908, 45, p. 834.

SUMMARY.

The natural complement content of human serum can be utilized in the serum test for syphilis if the serum be properly preserved and used when fresh. In addition the natural hemolysin against guinea-pig corpuscles can be employed in the hemolytic series in infants.

Theoretically the employment of human serum ought to give more constant results than those obtained by using foreign sera for complement and amboceptor, since the human amboceptor is more active in the presence of human complement than with that from the guinea-pig. The use of active serum obviates the destruction of the greater part of the so-called syphilitic antibody, thus rendering it possible to fix a larger quantity of complement than by other methods. That the heating of the tested serum to 56° C. for the purpose of destroying the proteotropic bodies is unnecessary when protein free antigens are used, such as that prepared by Noguchi, has been proved.

Practically any method utilizing the natural complement and hemolysin present in the serum is simpler, less expensive, and requires less time than other methods in vogue. The results obtained in over 600 tests have been uniformly trustworthy and have shown fewer errors than controls performed by the original Wassermann reaction and the Noguchi modification.

Of the published methods utilizing the natural complement and hemolysin that of Tschernogubow has proved the best, both practically and theoretically. Hecht's technic using sheep corpuscles and the hemolytic amboceptor against such corpuscles is good, but since the amboceptor content for sheep erythrocytes is proportionally less in amount than that for the red cells of the guinea-pig, when a similar number of cells are used, the complement unit necessary to complete hemolysis when guinea-pig corpuscles are employed is smaller, thus rendering the reaction more delicate, since no extra complement can be readily added. Thus a quantitative estimation of the intensity of the reaction can be carried out. For whereas in the Hecht method each 0.1 c.c. of serum contains only one unit of complement, the smallest quantity of antibody results in the fixation of a sufficient quantity

of complement to inhibit hemolysis: when guinea-pig corpuscles are employed, roughly six units of complement are present, rendering the estimation of the comparative strength of the reaction easy. For practical purposes a quantitative test which has proved efficient in the author's hands is the doubling of the number of corpuscles added, thus reducing the number of excess complement units to about two.

The objection frequently raised to methods such as the one here recommended is that both complement and hemolysin vary to such an extent that the reaction is worthless. In over 500 estimations of the combined action of complement and hemolysin the author has found less than three per cent showing variations of any demonstrable importance, most of these being accounted for by accidental deterioration of complement in handling. The variations in human complement appear less than those of guinea-pig complement. Variations in the amount of complementary body present, moreover, are much more readily controlled than when guinea-pig complement is used and in practical procedure can and must be determined for every serum at the time the test is performed.

Errors liable to give a false reaction, such as those occurring as a result of contamination of the serum with lipoids capable of binding the syphilitic antibody with the normal complement giving a negative result with positive sera, are more readily noted if the complement present in the serum tested be determined at the time the reaction is performed.

The fact that but very little agglutinin is present in human serum against guinea-pig corpuscles renders the reading of the reaction easier than by methods using an artificially induced amboceptor, since in rabbit serum a sufficiently large quantity of agglutinin is produced to clump the erythrocytes, rendering the identification of a partial hemolysis very difficult.

If serum to be tested is preserved in a uniform manner, preferably two hours in the incubator and subsequently in the ice chest, it will be found to maintain its complement content usually for 48 hours and always for 24 hours. Older serum should not be used.

Since the serum of infants cannot be examined by this method and since a certain number of sera will not be properly preserved

and for other reasons unfit for the reaction, other methods must be used. For this reason and also because the mere simplicity of the technic necessarily demands a more thorough knowledge of the underlying principles, it is the author's conviction that only those working in laboratories equipped for serum diagnosis of various kinds should be recommended to employ such a reaction.

For the testing of cases not adapted to examination by this method the author prefers the Noguchi system, utilizing quantities employed by Wassermann and with inactivated serum.